REMARKS/ARGUMENTS

Claims 1-3, 5-14 and 21-24 are active.

1.0 Support for Amendments

Part 2 of claims 1 and 21 has been revised in response to the Examiner's comment as regards the interpretation of step (2) of the claimed method (page 13, last line of and page 14, line 13 of the instant OA). Claims 1 and 21 have been modified to more clearly describe the yeast mitochondrial transformant of step (2). Support for the amendment can be found throughout the specification and drawings of the present application (see in particular page 9, line 27 to page 10, line 9; page 12, lines 17-20; page 17, lines 5-7 ; the example on page 26, line 33 to page 27 line 4 and figures 2-4). In addition, claim 3 has been restricted to a ΔSUV3 strain and claim 4 has been cancelled.

Figure 4 has been amended to correct an obvious inversion between the name of the two RNAs shown in the first and the second lane of the autoradiography. The resulting section corresponding to figure 4 (page 26, lines 33-37 of the present application) discloses that "*The signal with the RIP1 probe and the absence of signal with the COX1 probe demonstrate that the synthetic rho⁻ strain contain the RNA of interest, but no endogenous mitochondrial RNA (figure 4). By comparison, with the mitochondrial RNAs of the rho+ mit+ wild-type yeast strain, a signal is observed with the COX1 probe, but not with the RIP1 probe.*" Therefore, the signal shown in the first lane (normal mitochondria, *i.e.*, mitochondria of a rho+ mit+ wild-type yeast strain) corresponds to COX1 endogenous mitochondrial RNA, whereas the signal shown in the second lane (genetically modified mitochondria; synthetic rho⁻) corresponds to the RIP1 foreign RNA. This error, which would be immediately apparent to one of skill in the art from the disclosure, has been corrected in the new figure 4. Favorable consideration of this Amendment and allowance of this application are now respectfully requested.

2.0 Restriction/Election

The Applicants previously elected with traverse **Group I** claims 1-4, directed to producing a heterologous RNA of interest. The requirement has been made FINAL. The Applicants respectfully request that the claims of the nonelected group(s), or which are directed to other withdrawn subject matter, which depend from or otherwise include all the

limitations of an allowed elected claim, be rejoined upon an indication of allowability for the elected claim, see MPEP 821.04.

3.1 Preliminary remarks

The Applicant has noticed that no rejection was raised against claim 12. Therefore, the Applicant considers that in addition to claim 24, claim 12 (when rewritten in an independent form) would also be allowed.

The new claims clearly specify that the only DNA which is present in the mitochondria of the yeast mitochondrial transformant (step (2) of the claimed method) is the mitochondrial transcription vector (*i.e.*, the mitochondrial transformant is a synthetic rho$^-$ strain).

Therefore, the only RNAs that can be produced in the mitochondria of the synthetic rho$^-$ yeast strain are those encoded by the mitochondrial transcription vector, which are the heterologous RNA of interest only (when the mt transformation reporter gene is not transcribed) or the heterologous RNA of interest and the mt reporter gene RNA (when the mt transformation reporter gene is transcribed).

The mitochondrial RNA which is extracted (step (5) of the claimed method) contains one single RNA, the heterologous RNA of interest or two RNAs only (the heterologous RNA of interest and the mt reporter gene RNA), *i.e.*, isolated and substantially pure heterologous RNA. This is demonstrated in the example of the instant application (page 26, line 33 to page 27 line 4 and figure 4) which shows that the mitochondrial transformant of step (2) (synthetic rho- strain) are capable of specifically producing the heterologous RNA of interest in their mitochondria.

Therefore, the claimed method allows the specific production of a single heterologous RNA of interest, in isolated and pure form, *in vivo*, in yeast mitochondria lacking mt DNA (*ie.*, synthetic rho$^-$ strain).

The obviousness rejections of claims 1-11, 13, 14 and 21-23 based on Bonnefoy 2000, Bonnefoy 2000 in view of Bonnefoy 2001 or Bonnefoy 2001 in view of US 2009/0098653 which were raised in the previous OA and are maintained in the present OA cannot be sustained because the new claims are limited to a mitochondrial transformant lacking mtDNA (synthetic rho- strain). The claims do not include mitochondrial recombinants (rho$^+$ recombinant strain) and mtDNA as transforming DNA as explained in paragraph 3.2.1 and

3.2.2. In addition, claim 3 has been restricted to a ΔSUV3 strain and claim 4 has been cancelled, therefore US 2009/0098653 is not prior art for the claimed subject matter.

Contrary to the Examiner's assertion (page 3, line 21 to page 4, line 2; page 9, lines 5-6; page 14, lines 11-13 of instant OA), the prior art teaches that DNA expression in yeast mitochondria which implies DNA transcription (RNA production) occurs only in the presence of mtDNA, *i.e.*, in a rho$^+$ strain but not in a synthetic rho$^-$ strain (paragraph 3.2.3 and 3.2.4).

Therefore, it can only be through impermissible hindsight that heterologous RNA production in the mitochondria of a synthetic rho$^-$ strain seems obvious.

For these reasons, the combined teaching of the prior art document could not allow one skilled in the art to arrive at the claimed method. In addition the claimed method yields unexpected results as explained in paragraph 3.4.

3.2 Response to Examiner's arguments (pages 13-14 of the OA)

3.2.1 A mitochondrial recombinant (rho$^+$ recombinant or rho$^+$ mitochondrial transformant) is different from a mitochondrial transformant (synthetic rho$^-$ strain) according to the claimed method

The Examiner alleges (last paragraph of page 13) that the method of claim 1 includes mitochondrial recombinants because nothing in the claims precludes integration of the DNA once the mitochondria is transformed and also because the language in step (2) could be interpreted as including integration. The Applicants respectfully traverse this assertion.

The mitochondrial transformation (step (1) of the claimed method) is performed on a yeast cell that has no DNA in its mitochondria (yeast cell lacking mitochondrial DNA or rho^0 (ρ^0) strain). The mitochondrial transformation introduces the mitochondrial transcription vector into the mitochondria of the yeast cell that has no DNA in its mitochondria.

Therefore, as disclosed in the present application (page 12, lines 17-21), the only DNA that is present in the mitochondria of the yeast mitochondrial transformant (synthetic rho$^-$ strain) of step (2) is the mitochondrial transcription vector.

By contrast, as explained already in the response to the previous Office Action (definitions pages 8-9), a yeast mitochondrial recombinant is a yeast cell comprising a recombinant mitochondrial genome, that is a yeast cell which has a complete and functional mitochondrial genome (rho$^+$ strain) and has inserted (or integrated) transforming DNA into

the mitochondrial DNA (mtDNA), by homologous recombination (rho$^+$ (ρ^+) recombinant strain).

This fundamental difference between a mitochondrial transformant of a rho^0 yeast (synthetic rho$^-$ strain) and a mitochondrial recombinant of yeast (rho$^+$ recombinant strain) is explained in the definitions pages 5-7 of the specification and illustrated in figure 2 of the present Patent Application.

The rho$^+$ strains having integrated foreign DNA into mtDNA which are disclosed in Bonnefoy 2000 are called ρ+ mitochondrial transformants, according to the terminology used in Bonnefoy 2000 (page 1039, 2nd column, line 24 of the 1st paragraph) and Bonnefoy 2001 (figure 2; page 109, 2nd paragraph, line 5) or mitochondrial recombinants (rho$^+$ recombinant strain) according to the terminology used in the present Application and Bonnefoy 2001 (figure 3; page 105, 3rd paragraph, line 7; page 109, 4th paragaraph, line 2).

The incorporation of foreign DNA into the yeast mitochondrial genome requires that the foreign DNA is introduced in yeast mitochondria which have a mitochondrial DNA, *i.e*, the mitochondrial transformation with the foreign DNA is performed on a rho$^+$ strain.

The incorporation of foreign DNA into the yeast mitochondrial genome that leads to the production of a mitochondrial recombinant cannot occur when the foreign DNA is introduced in yeast mitochondria which have no DNA, *i.e*, the mitochondrial transformation with the foreign DNA is performed on a rho^0 strain.

Therefore, the method of claim 1 does not include mitochondrial recombinants because the mitochondrial transformation of a rho^0 strain in step (1) of the claimed method precludes integration of the DNA into the mitochondrial DNA once the mitochondria is transformed since a rho^0 strain has no DNA in its mitochondria.

Consequently, the language in step (2) cannot be interpreted as including integration because the mitochondrial transformation of a rho^0 strain in step (1) of the claimed method precludes integration of the DNA into the mitochondrial DNA once the mitochondria is transformed because rho^0 strains have no DNA in their mitochondria.

Step (2) can only be interpreted as identifying a yeast mitochondrial transformant which has mitochondria comprising the mitochondrial transcription vector (*i.e.*, a synthetic rho$^-$ strain).

3.2.2 The claims do not include mtDNA as the DNA being transformed

The Examiner alleges (last line of 2nd paragraph of page 14) that claim 1 includes mtDNA as the DNA being transformed.

The Applicant respectfully traverses this assertion. A mtDNA comprises multiple transcription units encoding the different mitochondrial rRNAs, tRNAs and proteins, as shown in figure 1 of the present Application for the yeast mtDNA. Yeast mtDNA is transcriptionnally active in yeast and all the RNAs (mRNAs, rRNAs and tRNAs) encoded by the mitochondrial genome are produced in yeast mitochondria. Therefore, mammalian mtDNA produces no RNA in (wild-type) yeast mitochondria but produces all the RNAs (mRNAs, rRNAs and tRNAs) encoded by the mammalian mt genome in mitochondria of genetically modified yeast strains.

By contrast, step (2) of claim 1 specifies that the only RNAs which are produced in the mitochondria of the synthetic rho$^-$ strain by said mitochondrial transcription vector, are the heterologous RNA of interest alone, when the reporter gene or the fragment thereof are not transcribed, or the heterologous RNA of interest and the transcript of the reporter gene or reporter gene fragment, when said reporter gene or fragment thereof are transcribed.

Furthermore, the present Application provides experimental evidence that production of one and only one specific RNA of interest, alone or with a mitochondrial transcription reporter gene transcript as with the claimed method, cannot be obtained when the transforming DNA is mtDNA (*i.e.*, full length mitochondrial genome). The example (page 26, line 33 to page 27 lines 4) and the corresponding figure (figure 4) of the present Application shows that the mitochondria of wild type yeast (rho+ strain) comprises the mitochondrial endogenous RNAs encoded by the mtDNA whereas the mitochondria of the mitochondrial transformants (synthetic rho- strain) from step (4) of the claimed method contains one RNA only which is the heterologous RNA of interest, *i.e.*, isolated or pure heterologous RNA. Therefore, claim 1 does not includes mtDNA as the DNA being transformed because, contrary to the claimed mitochondrial transcription vector, mtDNA is not capable of producing only the heterologous RNA alone when transformed into yeast mitochondria.

3.2.3 Bonnefoy et al., Mol. Gen. Genet., 2000, 262, 1036-1046 (Bonnefoy 2000)

The Examiner alleges that the only difference between Bonnefoy 2000 and the instant application is the isolation of the mitochondria (page 14, lines 3-4 of the instant OA).

The Applicants respectfully traverse this assertion for the reasons explained hereafter.

As explained already in the response to the previous Office Action, Bonnefoy 2000 disclose a method for analysing mitochondrial translation initiation (*i.e.*, protein synthesis) *in vivo* in *S. cerevisiae* yeast (Abstract, page 1036, lines 1-10), comprising:

(1) transforming the mitochondria of a ρ+ or ρ^0 yeast strain with a plasmid comprising the chimeric mt-reporter gene *cox2(1-91)::ARG8^m* (page 1040, 1st column, last paragraph; page 1039, 2nd column, 1st paragraph, lines 1-5 and 20-24), and only when the yeast is a ρ^0 strain,

(1bis) identifying mitochondrial transformants having incorporated said plasmid by mating with a (ρ+, mit$^-$) tester strain (page 1039, 2nd column, 1st paragraph, lines 5-11), and

(1ter) integrating the purified mitochondrial transformants into ρ+ mitochondrial genomes by cytoduction (page 1039, 2nd column, 1st paragraph, lines 11-16),

(2) identifying the ρ+ mitochondrial transformants having integrated said chimeric mt-reporter gene into mtDNA (page 1039, 2nd column, 1st paragraph, lines 16-20 and 24-28),

(3) culturing the ρ+ mitochondrial transformants,

(4) isolating total cellular RNA (page 1039, 2nd column, last paragraph to page 1040, 1st column, 1st paragraph and figure 4), and

(5) assaying mt-reporter mRNA expression (page 1039, 2nd column, last paragraph to page 1040, 1st column, 1st paragraph and figure 4).

Bonnefoy 2000 thus teach analysing mitochondrial translation by assaying mitochondrial transformation reporter gene expression in total RNA from a rho+ mitochondrial transformant which has mitochondria comprising a complete and functional mtDNA having integrated the mt reporter gene. The mt reporter gene RNA is produced in the yeast mitochondria, together with all the mtRNAs (mitochondrial mRNAs, rRNAs and tRNAs) encoded by the yeast mitochondrial genome. The mitochondria of the rho+ mitochondrial transformant thus contain all the mtRNAs (mitochondrial mRNAs, rRNAs and tRNAs) encoded by the yeast mitochondrial genome, in addition to the mt reporter gene RNA.

Contrary, to the Examiner's assertion (page 3, lines 13-14 of the instant OA) the plasmid which is used for the transformation does not carry an ARG8 reporter gene and cox2(1-91).

The plasmid which is used for the mitochondrial transformation comprises a chimeric mt reporter gene *cox2(1-91)::ARG8^{m}*, *i.e.*, one single gene which is a translational fusion of cox2(1-91) with ARG8^{m} (Abstract, page 1036, lines 1-10; figure 1A; Results, page 1040, 1st column, 1st and 2nd paragraphs). In addition, this chimeric mt-gene is a mitochondrial transformation reporter gene because its expression within mitochondria yields Arg8p which allows nuclear *arg8* mutants to grow without arginine (Abstract, page 1036, lines 6-10; Materials and methods, page 1039, 2nd column, 1st paragraph, lines 5-11 and 24-27; Results, page 1040, 1st column, 2nd paragraph).

In fact, The Examiner acknowledges that *ARG8^{m}* is an auxotrophic marker that is expressed in mitochondria and can be used to identify yeast mitochondrial transformants (page 3, lines 13-15 and 19-20 of the instant OA).

Therefore, Bonnefoy 2000 teach a synthetic rho^{-} yeast transformed with a vector comprising a mt transformation reporter gene. Bonnefoy does not teach a synthetic rho^{-} yeast transformed with a vector comprising a DNA encoding and heterologous RNA of interest and a mt transformation reporter gene according to step (2) of the claimed method.

The examiner alleges (page 3, line 21 to page 4, line 2 of the instant OA) that absent evidence to the contrary, the DNA encoding the RNA was under control of a promoter and terminator that are functional in yeast mitochondria, since the RNA was successfully produced in yeast mitochondria.

However, as explained already in the preceding paragraphs, Bonnefoy 2000 teach a mitochondrial transformation vector that does not contain a DNA encoding an heterologous RNA of interest but only a mt transformation reporter gene.

Therefore Bonnefoy 2000 do not teach heterologous RNA expression according to the claimed method. Bonnefoy teach only mt reporter RNA expression.

Furthermore, Bonnefoy 2000 teach that mt reporter gene expression which implies mt reporter RNA production occurs in the mitochondria of a ρ+ mitochondrial transformant (ρ+ recombinant) since mt reporter RNA expression analysis is performed on cellular RNA from a ρ+ recombinant (step 2 to 5 of the translation analysis method of Bonnefoy 2000).

Bonnefoy 2000 teach also (step 1bis of the method of Bonnefoy 2000) that the synthetic rho^{-} mitochondrial transformant which carries the mt reporter gene expression plasmid cannot be selected directly but only after crossing with a (ρ+, mit^{-}) tester strain which allows fusion of the mitochondria of the two strains and the integration of the mt reporter gene into the tester strain mtDNA by homologous recombination. Once integrated in

the mtDNA, the mt reporter gene is expressed, allowing the complementation of the mit⁻ allele of the reporter gene present in the tester strain and the identification of the rho⁻ mitochondrial transformant which correspond to the diploids having integrated the mt-reporter gene in their mtDNA.

Bonnefoy 2000 teach that "*A synthetic, recoded, version of* ***this gene ARG8m, can be*** *phenotypically* ***expressed as a reporter for mitochondrial gene expression when inserted into yeast mtDNA.***" (page 1037, 1st column, 2nd paragraph, lines 4-7).

Therefore, Bonnefoy 2000 teach that mt reporter gene expression which implies mt reporter RNA production does not occur in the mitochondria of a synthetic rho⁻ mitochondrial transformant (*i.e.*, in the absence of mtDNA) but only in the mitochondria of a ρ+ mitochondrial transformant (*i.e.*, in the presence of mtDNA).

It can only be through impermissible hindsight that heterologous RNA production in the mitochondria of a synthetic rho⁻ strain seems obvious.

The Examiner alleges that although Bonnefoy 2000 do not teach isolating mitochondria and then extracting RNA from the mitochondria, the end result of the instant application and Bonnefoy 2000 is the same because Bonnefoy 2000 teach extracting the total RNA which includes mitochondrial RNA (page 4, lines 2-3 and page 14, lines 1-6 of the instant OA).

However, total RNA includes mitochondrial RNAs and all the other cellular RNAs present in the cytoplasm and the nucleus. In addition, as explained already in the preceding paragraphs, Bonnefoy 2000 teach extracting total RNA from a rho+ yeast mitochondrial transformant which has mitochondria comprising a complete and functional mtDNA.

The mitochondria of the rho+ yeast mitochondrial transformant contain all the RNAs (mRNAs, rRNAs and tRNAs) encoded by the mitochondrial genome in addition to the mt-reporter gene RNA.

By contrast, the mitochondria of the synthetic rho- yeast of the claimed method contains only the heterologous RNA of interest alone or with the mt reporter gene RNA. Therefore, isolating mitochondria and then extracting RNA from the mitochondria according to the claimed method will result in RNA containing one single RNA or two RNAs only, the heterologous RNA of interest, alone or with the mt reporter gene RNA.

By contrast, extracting total RNA as taught in Bonnefoy 2000 will result in RNA containing all the RNAs (mRNAs, rRNAs and tRNAs) encoded by the mitochondrial genome and all other cellular RNAs present in the cytoplasm, in addition to the mt-reporter

gene RNA. Contrary to the Examiner's assertion, the end result of the instant application and Bonnefoy 2000 is totally different.

Conclusions:

Contrary to the Examiner's assertion (page 14, lines 3-4 of the instant OA), there are significant differences between the subject matter taught by Bonnefoy 2000 and the invention:

Bonnefoy 2000 do not teach a synthetic rho$^-$ yeast transformed with a vector comprising a DNA encoding and heterologous RNA of interest and a mt transformation reporter gene according to step (2) of the claimed method.

Bonnefoy 2000 teach a synthetic rho$^-$ yeast transformed with a vector comprising only a mt transformation reporter gene.

Bonnefoy 2000 teach to use a ρ+ mitochondrial transformant **but not** a synthetic rho$^-$ yeast to produce heterologous RNA in yeast mitochondria.

Bonnefoy 2000 do not teach isolating mitochondria from the yeast transformant and then extracting the RNA from said mitochondria (step 4 and 5 of the claimed method).

Therefore, the skilled artisan could not arrive at the claimed method that uses yeast lacking mitochondrial DNA transformed with a vector encoding a heterologous RNA of interest (synthetic rho- strain) to produce one isolated or pure heterologous RNA of interest in the yeast mitochondria.

In view of the teaching of Bonnefoy 2000, the skilled artisan would be motivated to produce a heterologous RNA of interest in the mitochondria of a ρ+ mitochondrial transformant having integrated the DNA encoding the heterologous RNA in mtDNA. The skilled artisan would have thus arrived at a different invention.

3.2.4 Bonnefoy et al., Meth. Enzymol., 2001, 350, 97 (Bonnefoy 2001)

As explained already in the response to the previous Office Actions, Bonnefoy 2001 teach how the mitochondrial genome can be manipulated using genetic transformation procedures which allow to deliver exogenous DNA into mitochondria and to create directed mutations or insert new genes into mtDNA via homologous recombination (introduction page 97). Bonnefoy 2001 is cited as teaching transforming yeast mitochondria with a vector carrying an ARG8 reporter gene (page 9, lines 4-5 of the instant OA).

The mitochondria transformation method is the one step or two-step biolistic transformation ("*Transformation of rho$^+$ cells with plasmids or linear DNA fragments*, page

109, 111; "*Delivery of DNA to mitochondrial compartment of rh^0 cells and detection of mitochondrial transformants*", page 101-109), *i.e.*, the same transformation method as in Bonnefoy 2000 (see section 3.2.3 above). Therefore, Bonnefoy 2001 teach:

(1) transforming the mitochondria of a ρ+ or ρ^0 yeast strain with a plasmid comprising the chimeric mt-reporter gene *ARG8^m*, and only when the yeast is a ρ^0 strain,

(1bis) identifying mitochondrial transformants having incorporated said plasmid by mating with a (ρ+, mit$^-$) tester strain, and

(1ter) integrating the purified mitochondrial transformants into ρ+ mitochondrial genomes by cytoduction

(2) identifying the ρ+ mitochondrial transformants having integrated said chimeric mt-reporter gene into mtDNA

Bonnefoy 2001 teach that novel mitochondrial phenotypes based on expression of foreign genes, like Arg+ prototrophy based on synthetic gene *ARG8^m* expression are generated **by placing the foreign gene into mtDNA** (last two paragraphs of page 98 and first paragraph of page 99; page 109, 2nd paragraph, lines 1-6).

Bonnefoy 2001 teach that synthetic rho$^-$ are useful to make mitochondrial recombinants because they "*allow **DNA introduced from outside the cell to be propagated within the organelle as a plasmid**, and the plasmid-borme mitochondrial sequences to recombine homologously with complete rho+ mtDNA.*" (first and last paragraph of page 100). Bonnefoy 2001 teach that the transforming vector carrying foreign DNA replicates in the synthetic rho$^-$ and the foreign DNA is then integrated into yeast mtDNA. There is no suggestion that expression of heterelogous RNA encoded by the plasmid would occur in the mitochondria of a synthetic rho$^-$ nor does this document provide any reasonable expectation of success that such expression would be useful or even that it would take place.

Therefore, Bonnefoy 2001 confirms what is taught by Bonnefoy 2000 (see paragraph 3.2.3), *i.e.*, that mt reporter gene expression which implies mt reporter RNA production does not occur in the mitochondria of a synthetic rho$^-$ mitochondrial transformant (*i.e.*, in the absence of mtDNA) but only in the mitochondria of a ρ+ mitochondrial transformant (*i.e.*, in the presence of mtDNA).

Conclusions:

Contrary to the Examiner's assertion (page 11, lines 8-9; page 14, lines 11-12 of the instant OA), there are significant differences between the subject matter taught by Bonnefoy 2001 and the invention:

Bonnefoy 2001 do not teach a synthetic rho$^-$ yeast transformed with a vector comprising a DNA encoding and heterologous RNA of interest and a mt transformation reporter gene according to step (2) of the claimed method. Bonnefoy 2001 teach a synthetic rho$^-$ yeast transformed with a vector comprising only a mt transformation reporter gene.

Bonnefoy 2001 teach to use a rho+ mitochondrial transformant **but not** a synthetic rho$^-$ yeast to express a foreign DNA in yeast mitochondria.

Bonnefoy 2001 do not teach production of RNA in yeast transformant as acknowledged by the Examiner (page 9, end of 2nd paragraph of instant OA). However foreign DNA expression in mitochondria implies the production of the corresponding heterologous RNA.

Bonnefoy 2001 do not teach isolating mitochondria from the yeast transformant and then extracting the RNA from said mitochondria (step 4 and 5 of the claimed method), as acknowledged by the Examiner (page 9, end of 2nd paragraph of instant OA).

Therefore, the skilled artisan could not arrive at the claimed method that uses yeast lacking mitochondrial DNA transformed with a vector encoding a heterologous RNA of interest (synthetic rho- strain) to produce one isolated or pure heterologous RNA of interest in the yeast mitochondria.

In view of the teaching of Bonnefoy 2001, the skilled artisan would be motivated to produce a heterologous RNA of interest in the mitochondria of a rho+ mitochondrial transformant having integrated the DNA encoding the heterologous RNA in mtDNA. The skilled artisan would have thus arrived at a different invention.

3.3 Claim rejections under 35 USC § 103 (a) (pages 2-13 of instant OA)

3.3.1 Non-obviousness of claims 1, 2, 5, 8-10, 21, 22 and 23 over Bonnefoy 2000 in view of Dekker et al. (Curr. Genet., 23, 22-27, 1993)

Claims 1, 2, 5, 8-10, 21, 22 and 23 were rejected under 35 USC 103(a) as being unpatentable over Bonnefoy 2000, in view of Dekker *et al.* Bonnefoy 2000 is discussed above in paragraph 3.2.3.

Dekker *et al.*, was cited as disclosing isolating yeast mitochondria and extracting mRNA (page 4, lines 4-5 of instant OA).

Dekker *et al.*, teach (Material and methods, page 23, paragraphs 1 to 4) isolating mitochondria from wild-type (rho+) *S. carlsbergensis* yeast and purifying ribosomes, mitochondrial tRNAs, and an extract having tRNA acylation and translation elongation

activity. Dekker *et al.* teach also producing mt RNA (COX2) by *in vitro* transcription with SP6 RNA polymerase of pCOX2Δ9 (end of 3rd paragraph of Materials and methods).

There is no suggestion to select a synthetic rho⁻ mitochondrial transformant to express one heterologous RNA of interest (not encoded by the mt genome) in the mitochondria of the synthetic transformant, nor does this reference provide any expectation of success that such expression would be useful or even that it would take place.

The Examiner considers that the ordinary skilled artisan would have been motivated to combine because Dekker *et al.* teach that isolating mitochondrial mRNA makes it possible to study translation in a homologous system (page 4, lines 6-8 of the instant OA). However, contrary to the Examiner's assertion, Dekker *et al.*, do not do not teach isolating yeast mitochondria and extracting mRNA.

Dekker *et al.* teach isolating yeast mitochondria and producing mRNA by *in vitro* transcription. Furthemore, Dekker *et al.*, teach an *in vitro* translation system for yeast mitochondrial mRNAs which does not work, as disclosed in Dekker et al. (end of summary, page 22; end of introduction, page 23) and confirmed in Bonnefoy 2000 (introduction, page 1036, first line): "*The mechanisms that control translation initiation in mitochondrial systems are poorly understood, owing to the deficiencies of organelle-derived in vitro translation systems (Dekker et al., 1993).*" Therefore, the ordinary skilled artisan would have been dissuaded to combine the teachings of Bonnefoy 2000 with Dekker *et al.* because the *in vitro* translation system disclosed in Dekker does not work as opposed to the *in vivo* translation system disclosed in Bonnefoy 2000 which works.

Assuming *arguendo* that the skilled artisan would have used the rho+ mitochondrial transformant of Bonnefoy 2000 in the *in vitro* translation assay of Dekker *et al.*, he would have isolated the mitochondria and isolated the mitochondrial tRNAs, while he would have produced the mt RNA using an *in vitro* transcription system.

Mitochondrial tRNAs is a mixture of RNAs encoded by the mtDNA which is different from the isolated or pure heterologous RNA (not encoded by the yeast mt DNA, as defined page 5, line 28-30 of the present Application) that is produced by the claimed method. Therefore, by combining the teachings of Bonnefoy 2000 with Dekker *et al.*, the skilled artisan would arrive at a different invention.

The ordinarily skilled artisan could not have arrived at the invention because the prior art do not disclose or suggest the combination of step (1) to (5) of the claimed method for producing one heterologous RNA of interest (not encoded by the yeast mtDNA), or provide a

reasonable expectation of success that such production of one isolated or pure heterologous RNA would take place because heterologous RNA production in the mitochondria of a synthetic rho⁻ strain was not obvious for the reasons explained before (paragraph 3.2.3). It can only be through impermissible hindsight that heterologous RNA production in the mitochondria of a synthetic rho⁻ strain seems obvious. For these reasons, this rejection cannot be sustained.

3.3.2 Non-obviousness of claim 3 over Bonnefoy 2000 and Dekker et al., and further in view of Dziembowski et al.

Claim 3 was rejected under 35 USC 103(a) as being unpatentable over Bonnefoy 2000 and Dekker *et al.*, as applied to claims 1, 2, 5, 8-10, 21, 22 and 23, and further in view of Dziembowski *et al.*, JBC, 2003, 278, 1603-1611. This rejection is not sustainable over the combination of Bonnefoy 2000 and Dekker for the reasons discussed above in paragraph 3.3.1

Furthermore, Dziembowski does not suggest all the elements missing from the two primary references, such as selecting a yeast lacking mitochondrial DNA transformed with a vector encoding a heterologous RNA of interest (synthetic rho- strain) to produce one isolated or pure heterologous RNA of interest (not mRNA), *in vivo*, in the yeast mitochondria, nor does this reference provide a reasonable expectation of success that such production would be useful or even that it would take place, as discussed already in the response to the previous Office Actions. Accordingly, this rejection cannot be sustained.

3.3.3 Non-obviousness of claim 4 over Bonnefoy 2000 and Dekker et al., and further in view of Komiya et al. and Hwang et al.

Claim 4 was rejected under 35 USC 103(a) as being unpatentable over Bonnefoy 2000 and Dekker *et al.*, as applied to claims 1, 2, 5, 8-10, 21, 22 and 23, and further in view of Komiya *et al.*, JBC, 1964, 269, 30893-3087 and Hwang *et al.*, J. Virol., 2000, 74, 4074-4084. This rejection is not sustainable over the combination of Bonnefoy 2000 and Dekker for the reasons discussed above in paragraph 3.3.1

Furthermore, these secondary references do not suggest all the elements missing from the two primary references, such as selecting a yeast lacking mitochondrial DNA transformed with a vector encoding a heterologous RNA of interest (synthetic rho- strain) to produce one isolated or pure heterologous RNA of interest (not mt RNA), *in vivo*, in the yeast mitochondria, nor does this reference provide a reasonable expectation of success that such

production would be useful or even that it would take place, as discussed already in the response to the previous Office Actions. Accordingly, this rejection cannot be sustained.

3.3.4 Non-obviousness of claim 6 over Bonnefoy 2000 and Dekker et al., and further in view of Anziano et al.

Claim 6 was rejected under 35 USC 103(a) as being unpatentable over Bonnefoy 2000 and Dekker *et al.*, as applied to claims 1, 2, 5, 8-10, 21, 22 and 23, and further in view of Anziano *et al.*, Proc. Natl. Acad. Sci. USA, 1991, 88, 5592-5596. This rejection is not sustainable over the combination of Bonnefoy 2000 and Dekker for the reasons discussed above in paragraph 3.3.1 Furthermore, this secondary reference does not suggest all the elements missing from the two primary references, as discussed already in the response to the previous Office Actions. Accordingly, this rejection cannot be sustained.

3.3.5 Non-obviousness of claim 13 over Bonnefoy 2000 and Dekker et al., and further in view of Kim et al.

Claim 13 was rejected under 35 USC 103(a) as being unpatentable over Bonnefoy 2000 and Dekker *et al.*, as applied to claims 1, 2, 5, 8-10, 21, 22 and 23, and further in view of Kim *et al.*, Cancer Res., 1997, 57, 3115-3120. This rejection is not sustainable over the combination of Bonnefoy 2000 and Dekker for the reasons discussed above in paragraph 3.3.1 Furthermore, this secondary reference does not suggest all the elements missing from the two primary references, as discussed already in the response to the previous Office Actions. Accordingly, this rejection cannot be sustained.

3.3.6 Non-obviousness of claim 14 over Bonnefoy 2000 and Dekker et al., and further in view of Dziembowski et al. and di Rago et al.

Claim 14 was rejected under 35 USC 103(a) as being unpatentable over Bonnefoy 2000 and Dekker *et al.*, as applied to claims 1, 2, 5, 8-10, 21, 22 and 23, and further in view of Dziembowski *et al.* and di Rago *et al.*, JBC, 1988, 263, 12564-12570. This rejection is not sustainable over the combination of Bonnefoy 2000 and Dekker for the reasons discussed above in paragraph 3.3.1. Furthermore, these secondary references do not suggest all the elements missing from the two primary references, as discussed already in the response to the previous Office Actions. Accordingly, this rejection cannot be sustained.

3.3.6 Non-obviousness of claims 1, 2, 5, 8-10, 21, 22 and 23 over Bonnefoy 2001 in view of Bonnefoy 2000 and Dekker et al. (Curr. Genet., 23, 22-27, 1993)

Claims 1, 2, 5, 8-10, 21, 22 and 23 were rejected under 35 USC 103(a) as being unpatentable over Bonnefoy 2001, in view of Bonnefoy 2000 and Dekker *et al.* This rejection is not sustainable over the combination of Bonnefoy 2000 and Dekker for the reasons discussed above in paragraph 3.3.1. Bonnefoy 2001 is discussed in paragraph 3.2.4. Bonnefoy 2001 fails to compensate the lack of teaching from Bonnefoy 2000 and Dekker because heterologous RNA production in the mitochondria of a synthetic rho$^-$ strain was not obvious for the reasons explained before (paragraph 3.2.4). Accordingly, this rejection cannot be sustained.

3.3.7 Non-obviousness of claim 3 over Bonnefoy 2001 and US 2009/0098653, and further in view of Dziembowski et al.

Claim 3 stands rejected under 35 USC 103(a) as being unpatentable over Bonnefoy 2001 and US 2009/0098653 and further in view of Dziembowski et *al.* Claim 3 has been restricted to a ΔSUV3 strain. US 2009/0098653 is not prior art for the new claim 3. Bonnefoy 2001 is discussed in paragraph 3.2.4. The rejection is not sustainable over the combination of Bonnefoy 2001 and Dziembowski. The combined teachings of Bonnefoy 2001 and Dziembowski do not disclose or suggest the combination of step (1) to (5) of the claimed method for producing one heterologous RNA of interest (not encoded by the yeast mtDNA), or provide a reasonable expectation of success that such production of one isolated or pure heterologous RNA would take place because heterologous RNA production in the mitochondria of a synthetic rho$^-$ strain was not obvious for the reasons explained before (paragraph 3.2.4 and 3.3.2). Accordingly, this rejection cannot be sustained.

3.3.8 Non-obviousness of claim 4 over Bonnefoy 2001 and US 2009/0098653, and further in view of Komiya et al. and Hwang et al.

Claim 4 has been cancelled rendering this objection moot.

3.4 The claimed method yields suprising and superior results

Contrary the methods of the prior art which yields a mixture of mitochondrial RNAs and heterologous RNA of interest, the claimed method allows to produce the RNA of interest in large amounts, for a low cost, and in a form which is stable and can be readily isolated insofar as the only RNAs which are produced in the mitochondria of the synthetic rho$^-$ yeasts

are the RNA of interest alone or with the RNA of the mitochondrial transformation reporter gene (paragraphs 3.1, 3.2.2 and 3.2.3).

The prior art teaches only that mitochondrial genes (yeast *COX2*) or yeast nuclear genes (*ARG8*^{m}) encoding proteins which are normally imported into to the mitochondria (Arg8p) can be expressed in yeast mitochondria. Therefore, it was not unexpected that any DNA of interest (not related to mitochondria) could be expressed in mitochondria.

Furthermore, the prior art teaches only that mitochondrial transcription occurs in the mitochondria of rho^{+} yeast cells (comprising an intact and functional mitochondrial DNA or mitochondrial DNA carrying local alteration in its sequence which inactivate the respiratory function of mitochondria (rho^{+} mit^{-}). Therefore, it was surpising that transcription of any DNA sequence of interest (not related to mitochondria) could occur efficiently *in vivo* the mitochondria of synthetic rho^{-} yeast which lack all the mitochondrial DNA except the DNA encoding the mitochondrial transformation reporter gene.

It was not obvious also that the heterologous RNA produced in the mitochondria of such synthetic rho^{-} yeast could be stable. Therefore, as discussed above this evidence shows that the claimed method yields surprising and superior results.

Conclusion

In view of the amendments and remarks above, the Applicants respectfully submit that this application is now in condition for allowance. An early notice to that effect is earnestly solicited.

Respectfully submitted,

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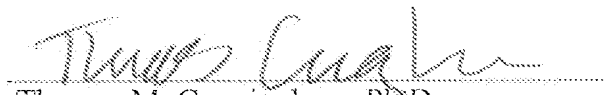

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